STRUCTURAL REQUIREMENTS OF THE GDP BINDING SITE OF ELONGATION FACTOR Tu

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1. Introduction

Several translation factors of bacterial protein biosynthesis require GTP to perform their function on the ribosome [2]. While much has been found about the specificity of the interaction of translocation factor G [3–5] in respect to its uncoupled GTPase activity and its complex formation with ribosomes, much less information is available about the structural requirements of the GDP binding site of elongation factor Tu. Hamel [6] and Hamel and Cashel [7] found that the natural and synthetic nucleotide analogues pppGpp, dGTP, 3′dGTP and 3′dNH$_2$GTP were similar to GTP in supporting *EF-Tu-dependent *N*-acetyl–Phe–Phe–tRNA formation, whereas ox GTP, ox-red GTP and ITP were only weakly effective. The corresponding diphosphates ox GDP, ox-red GDP and IDP were found to complex with EF-Tu by the nitrocellulose filter assay somewhat in contrast to the results of Gordon and Bodley [8]. We have used some of these and other nucleoside diphosphates with differences in the base and ribose moieties and tested their ability to replace GDP in binding to EF-Tu and thereby obtained a quantitative measure of their relative binding constants.

2. Materials and methods

EF-Tu from *E. coli* (MRE 6000) was isolated by methods similar to those described by Arai et al. [9]. The protein had a molecular weight of 44 000 ± 2000 and had a specific binding activity of 20 000 ± 2000 pmol/mg. EF-Tu from *Bacillus stearothermophilus* was isolated as previously described [10] and had a specific binding activity of 18 000 pmol/mg. ADP, GDP, IDP, XDP, and UDP were purchased from Pharma Waldhof (Mannheim), dGDP and dTDP were from Sigma, G$^{2'}_{m}$DP from PL Biochemicals and [^{3}H]-GDP (5 Ci/mmol) from Amersham Buchler. Preparation of ox GDP and ox-red GDP was according to Gordon and Bodley [8], h^{6}GDP was a gift from Dr H. Scheit, Göttingen. Br^{8}GDP was prepared by brominating GDP according to Ikehara and Uesugi [11], m^{7}GDP was obtained by methylation of GDP with dimethyl sulphate, at pH 4.4, as described by Haines et al. [12], s^{6}GDP was prepared by phosphorylating 6-thioguanosine (Waldhof) according to published procedures [4]. All synthesized GDP analogues were purified by binding them in solution, at pH 8.0, to a column of

*Abbreviations: EF-Tu (BS), EF-Tu (EC) are the elongation factors Tu from *Bacillus stearothermophilus* and *Escherichia coli*, respectively. In general, the one letter nomenclature for mononucleotides was used [1]. The substituents br^8, s^6, m^7 being 8-bromo, 6-thio, 7-methyl. h^{6}GDP is 2-amino purine ribonucleoside-5′-diphosphate, ox GDP the dialdehyde derivative of GDP obtained by periodate oxidation and ox-red GDP the dialcohol derivative of GDP obtained by periodate oxidation and borohydride reduction, G$^{2'}_{m}$DP is 2′-*O*-methyl-GDP.

DE-52 (Whatman) pre-equilibrated at pH 7.5, in the bicarbonate form and eluting with a linear-gradient of 0.04–0.4 M triethylammonium bicarbonate. All nucleotides used ran as one spot by TLC on PEI cellulose sheets (Polygram Cel-300, Machere and Nagel) in 0.75 M KH_2PO_4, pH 3.4 and/or 1 M LiCl. The nucleotides were identified and their concentrations determined by ultraviolet absorption. Nitrocellulose filters were from Schleicher and Schuell (BA85, 0.45 μm).

3. Results and discussion

EF-Tu (EC) or EF-Tu (BS), 1.8×10^{-7} M in 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 0.5 mM dithioethrytol, freshly diluted from concentrated stock solutions, was incubated for 2 h at room temperature with approximately 10^{-6} M [^{3}H]GDP and an unlabelled nucleotide, the concentration of which ranged from 10^{-8}–10^{-3} M. The samples were filtered and the amount of radioactivity bound to the nitrocellulose filter measured. The percentage of radioactivity bound relative to control without added nucleotide was plotted against nucleotide concentration. Some of the results, obtained with EF-Tu (BS) and a small number of nucleotides, are shown in fig.1, and are seen to be typical sigmoidal titration-curves. The position of the curve along the abscissa for any

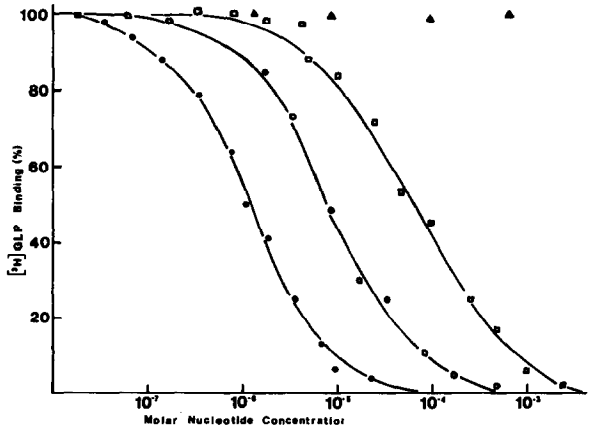

Fig.1. Inhibition of [^{3}H]GDP binding to EF-Tu by varying concentrations of nucleotide. (●) GDP, (○) dGDP, (□) IDP, (▲) ADP.

Table 1
Relative concentrations (to GDP) required to displace 50% of [^{3}H]GDP bound to EF-Tu

Compound	EF-Tu (BS)	EF-Tu (EC)
GDP	1.0	1.0
$G^{2'}_{m}DP$	0.26	0.23
dGDP	8.3	–
br^8GDP	26	–
ox GDP	50	59
XDP	67	–
IDP	71	71
UDP	130	–
s^6GDP	240	–
ox-red	420	420

nucleoside diphosphate can be used to measure its affinity for EF-Tu. These positions were measured at the midpoints of the titration curves (i.e., where the amount of bound [^{3}H]GDP was 50% of the maximal value) and compared to the value obtained with unlabelled GDP. In table 1 the ratios of the GDP midpoint to those of the nucleoside diphosphates with any measurable affinity are presented. Some of the nucleotides were also tested with EF-Tu (EC) and the results show, within the limits of accuracy, the same pattern of affinity. The nucleotides h^6GDP, m^7GDP, ADP, CDP and dTDP shown no affinity for the GDP binding site of EF-Tu.

As can be seen, the binding of GDP to EF-Tu is very sensitive to changes in both the ribose and the base moieties of the nucleotide. Thus the methylation of the 2′-hydroxyl group leads to an increase, while the replacement of this hydroxyl group by hydrogen leads to a decrease in binding affinity. This is in contrast to the results of Hamel and Cashel [7] who find no difference between GTP and dGTP measured in partial reactions of protein biosynthesis. Opening of the ribose ring leads to analogues with decreased binding affinity, where ox GDP is a better analogue than ox-red GDP. Here again, the ratio of apparent K_m values of ox-red GTP versus GTP in the EF-Tu dependent *N*-acetyl–Phe–Phe–tRNA formation [6] is much higher than the ratios of ox-red GDP and IDP versus GDP binding affinities, which could indicate that GDP and GTP binding on EF-Tu have different structural requirements. The binding of ox GDP and ox-red GDP has not been detected by Gordon and Bodley [8].

Modification at various positions of the base moiety leads to a rather drastic decrease in binding ability. Our results show that both the oxygen in the 6-position and the amino group in the 2-position are important for binding. However, the effect of the former is more pronounced, since its replacement with sulphur in s^6GDP leads to a very weak binding and the replacement with hydrogen (h^6GDP) leads to no binding, while nucleotides without a 2-amino group (XDP, IDP) are inhibitors of GDP binding. The interaction of the 6-carbonyl with the protein is, however, not a Schiff's base type interaction, since [^{3}H]GDP cannot be irreversibly bound to the protein by reduction with sodium borohydride (unpublished observation). It is interesting to note that the derivative with the rather bulky bromo group, br^8GDP, is a good inhibitor of GDP binding, which could indicate that the 8 position of the purine ring is a possible choice for introduction of 'reporter groups' into the GDP molecule.

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